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STUDIES ON THE REPRODUCTION OF THREE SPECIES OF *PEROPHORA* (ASCIDIACEA)*

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ABSTRACT

The breeding season is summer for *Perophora formosana* and *P. sagamiensis*, and winter for *P. japonica*. In all species, the gonads lie on the left side within the first intestinal loop. Eggs at the bottom of the ovary mature first. Mature eggs are ovulated into the postero-dorsal corner of the right peribranchial cavity, where they gain access to the brood pouch. In *P. sagamiensis*, the branchial basket and digestive tract of a zooid disintegrate at the onset of brooding, and after sexual reproduction the whole zooid is resorbed. Very often, such degenerating zooids are replaced by new zooids budded from the basal stolon.

A new mode of asexual reproduction, termed "terminal budding," was found in *P. japonica*. The distal end of a stolon rises up off the substratum and develops into a discoidal stellate reproductive body which is gorged with granular amoebocytes. This terminal bud is separated from the colony, then is carried away by water currents and adheres to a new substratum, on which it elongates stolons to found a new colony. Terminal budding can coexist with ordinary subterminal budding in the same stolon, but it is suppressed by sexual reproduction.

P. formosana is considered more primitive than the other two species.

INTRODUCTION

The genus *Perophora* consists of colonial ascidians, familiar to most marine biologists. In the colony, all the zooids are connected basally by branched creeping stolons from which they have arisen as buds. Generally, budding occurs successively at a short distance from the tip of a growing stolon. The stolon contains a vertical mesenchymatous septum which divides the lumen into two channels. This septum disappears near the distal end of the stolon allowing continuity of blood flow between the right and left channels. *Ecteinascidia* is another colonial ascidian closely allied to *Perophora*. These two genera constitute the family Perophoridae (Van Name, 1945; Berrill, 1950).

Asexual reproduction of *Perophora* has been the subject of many studies over a long period of time. The course of blastogenesis was studied by Kowalevsky (1874), Ritter (1896), Lefèvre (1898), Brien and Brien-Gavage (1927), and Deviney (1934). Freeman (1965) showed experimentally that the lymphocyte, a type of blood cell, participates in the initiation of blastogenesis. The behavior of isolated stolon pieces or zooid-stolon preparations under various external conditions has been observed by Huxley (1921), Goldin (1948), Barth and Barth (1966), and Fukumoto (1971). Sexual reproduction of *Perophora* has received comparatively little attention. All species of *Perophora* are ovoviviparous; embryogenesis takes place in a brood pouch.

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The activities and structure of the free-swimming larva were studied by Grave and McCosh (1923), and some aspects of metamorphosis by Oka (1960) and Terakado (1967). Recently, Fukumoto (1981) described the ultrastructural features of the sperm. As to *Ecteinascidia*, the origin of gonads and sex cells was studied by Simkins (1924) and blastogenesis by Plough and Jones (1939).

We have been rearing three species of *Perophora* for these several years, and have studied their sexual and asexual reproduction revealing some new facts. Comparative morphology of their gonads and brood pouches, and two new modes of asexual reproduction, called "terminal" and "replacement" budding, constitute the major contents of this paper.

MATERIALS AND METHODS

The following three Japanese species of *Perophora* were used: *P. formosana* (Oka), *P. japonica* Oka, and *P. sagamiensis* Tokioka. For taxonomical remarks of these species, see Tokioka (1953).

Some colonies of *P. formosana* were originally collected at Shimoda (Shizuoka Prefecture), those of *P. japonica* in the vicinity of the Asamushi Marine Biological Station (Aomori Prefecture), and those of *P. sagamiensis* in the vicinity of the Seto Marine Biological Laboratory (Wakayama Prefecture). They were then reared in a small bay to which the Shimoda Marine Research Center is facing. All colonies were grown on glass plates which were set in boxes hung below the sea's surface. When necessary, the plates with the colonies attached were brought into the laboratory, and were observed under a stereomicroscope.

Observations on living material were supplemented with histological study. For this purpose, pieces of colonies were fixed in Bouin's fixative made in sea water. They were dehydrated through a graded series of ethanols; and paraffin sections, 7 μm thick, were stained with Delafield's hematoxylin and eosin.

RESULTS

Sexual reproduction: external observations

The breeding season of *P. formosana* and *P. sagamiensis* is summer, with the highest activity during July and August; that of *P. japonica* is winter, with the peak period during December and January.

Perophora is hermaphroditic like other ascidians, and is more or less protandrous. Both the testis and the ovary lie within the first intestinal loop, in the mantle on the left side of the body. The sperm duct accompanies the rectum and opens into the cloacal cavity shortly anterior to the anus. Embryogenesis takes place within the brood pouch located in the right peribranchial cavity. The oviduct leading to the brood pouch is very hard to demonstrate in live or fixed total specimens. Anatomical details of the gonads and the brood pouch are different from species to species.

In *P. formosana*, the testis is a large oval mass located near the ventral corner of the intestinal loop. The ovary is located on the sperm duct at some distance from the testis (Fig. 1A). The brood pouch is a tubular organ, extending dorso-ventrally in the posterior portion of the right peribranchial cavity (Fig. 1B). The lumen of the brood pouch communicates with the peribranchial cavity through the ventral or distal opening. As ovulation proceeds, the brood pouch grows in length distalward. Ovulation continues in a chronological order, so that embryos are naturally arranged in a series with the earliest in developmental stage at the proximal end of the pouch

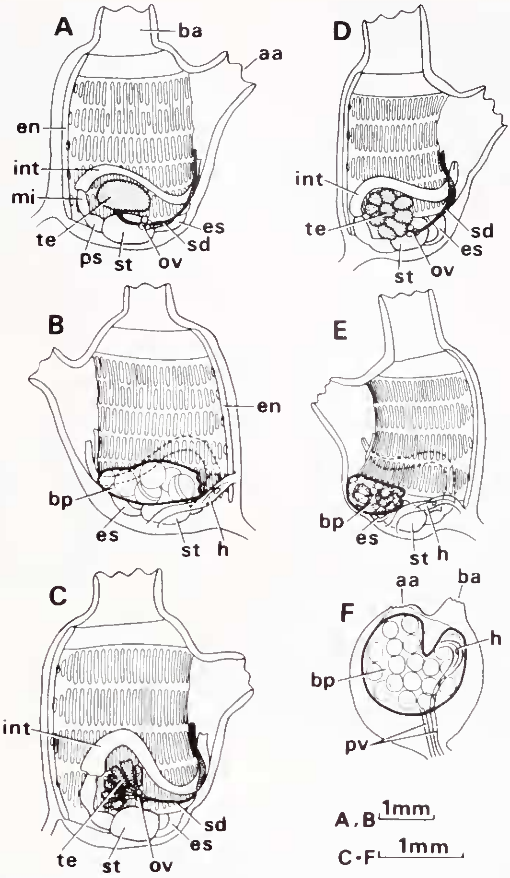
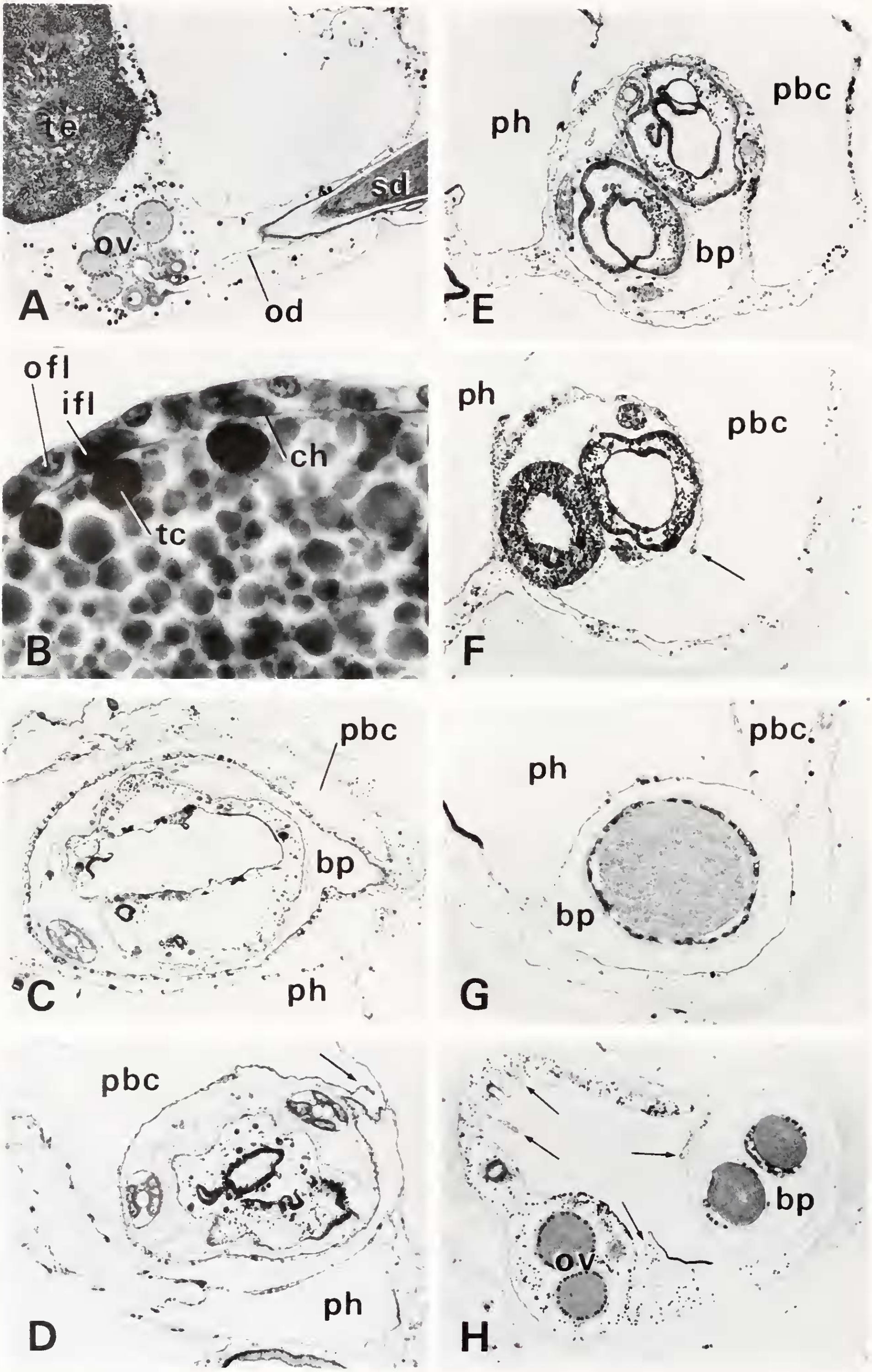


FIGURE 1. External views of zooids, showing gonads and brood pouch. All figures were drawn from living specimens with the aid of a camera lucida. A: *P. formosana,* from left side. B: *P. formosana,* from right side. C: *P. japonica,* from left side. D: *P. sagamiensis,* from left side. E: *P. sagamiensis,* from right side. F: A sac-like zooid of *P. sagamiensis,* crowded with embryos after the resorption of the viscera, from right side. aa, atrial aperture; ba, branchial aperture; bp, brood pouch with embryos; en, endostyle; es, esophagus; h, heart; int, intestine; mi, mid-intestine; od, oviduct; ov, ovary (immature); ps, post-stomach; pv, peduncular vessels; sd, sperm duct; st, stomach; te, testis (fully grown).

and the most advanced at the distal end. Less than ten embryos develop within the pouch at one time. As soon as the tadpole stage is completed, the embryos leave the brood pouch and then the mother zooid through its atrial aperture as free larvae.

In *P. Japonica,* the testis is divided into 6–10 wedge-shaped follicles which are arranged fanwise. Small sperm ducts issuing from respective follicles are united at the rivet of the testicular fan to form a common sperm duct. The ovary is located at the starting point of the common sperm duct (Fig 1C). The brood pouch of this species is quite similar in appearance to that of *P. formosana.* More than 10 embryos are found at a time in a zooid. Nearly mature embryos are often pushed out of the brood pouch into the peribranchial cavity. They soon hatch and escape from the zooid through its atrial aperture as free-swimming larvae.

In *P. sagamiensis,* the testis is subdivided into several irregular lobes Tokioka (1953) reported in the original description of this species that the testis is a large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oval mass. In all probability, this is an error. Our material agrees with Tokioka's description of the zooid except for the testis. As in *P. japonica*, the ovary is located close to the testis (Fig. 1D). The brood pouch initially appears as a small sac-like organ near the postero-dorsal corner of the right peribranchial cavity as in *P. formosana* and *P. japonica;* but unlike these species, the brood pouch of *P. sagamiensis* is situated below the branchial basket or pharynx (Fig. E). A network of blood lacunae is conspicuous in the wall of the brood pouch. At about the time when the brood pouch contains a few embryos, the mother zooid closes its branchial and atrial apertures, the former permanently and the latter temporarily, until the liberation of larvae. Within the zooid, the branchial basket and digestive tract disintegrate and are fully resorbed; concomitantly, the brood pouch augments more and more occupying the cavity formed by the disappearance of the branchial basket. Thus, after ovulation of all eggs, the zooid becomes a closed sac filled with growing embryos, about 20 in number (Fig. 1F). The heart continues to beat even in this condition. When embryos develop into mature tadpoles, they swim out through the now slightly opened atrial aperture. Following the liberation of all larvae, the empty sac of the mother zooid shrinks and is eventually resorbed into the basal stolon.

Sexual reproduction: histological observations

The zooids of *P. formosana* and *P. japonica* are protandrous. Mature sperm appear in the testis somewhat prior to the onset of ovulation. Subsequently, the testis continuously diminishes in size though it contains some sperm almost throughout the period of ovulation. On the other hand, each zooid of *P. sagamiensis* is definitely protandrous (Fig. 2A). The testis sheds all the sperm prior to ovulation, or at least before the onset of the involution of the branchial basket. In all species, the sperm duct consists of cuboidal cells and is sparsely ciliated, except near the distal end where it is heavily ciliated.

The following description of the ovary is common to all species studied. A young ovary is essentially an oval vesicle consisting of squamous epithelial cells and small previtellogenic oocytes. Towards the blind end of the ovary, oocytes are seen to become successively larger (Fig. 2A); this fact was illustrated with *Perophora listeri* by Kowalevsky (1874). These small oocytes are surrounded by a single layer of primary follicular cells and are embedded in the ovarian wall. As they grow larger, they protrude outward accompanied by individual short follicle stalks which are continuations of the ovarian wall. A mature oocyte is surrounded by a pair of follicular layers, the outer and the inner, both consisting of squamous or somewhat cuboidal cells, and a thin non-cellular layer known as the chorion (Fig. 2B). Beneath the chorion, the so-called test cells are scattered; their cytoplasm is filled with intensely basophilic globules. The oocyte is polylecithal and contains numerous eosinophilic yolk granules. The oviduct consists of a single layer of squamous epithelial cells and is sparsely ciliated. The oviduct, which lies more superficially than the

FIGURE 2. Sections of sexual reproductive organs. A: *P. sagamiensis*, section through an immature ovary and the sperm duct. ×90. B: *P. japonica*, part of a mature egg. ×1000. C: *P. formosana*, cross section through a proximal part of the brood pouch. ×70. D: *P. formosana*, cross section through a distal part of the brood pouch. Arrow indicates the free edge of the wall of brood pouch. ×70. E: *P. japonica*, cross section through a proximal part of the brood pouch. ×70. F: *P. japonica*, cross section through a distal part of the brood pouch. Arrow indicates the free edge of the wall of brood pouch. ×70. G: *P. sagamiensis*, cross section of an early brood pouch. ×100. H: *P. sagamiensis*, a zooid at an early stage of brooding. Arrows indicate remnants of the branchial basket. ×40.bp, brood pouch; ch, chorion; ifl, inner follicular layer; od, oviduct; ofl, outer follicular layer; ov, ovary; pbc, peribranchial cavity; ph, pharynx; sd, sperm duct; tc, test cell; te, testis.

sperm duct, accompanies the proximal half of the sperm duct but fails to follow its distal curve; the oviduct extends to and beyond the middorsal line to open in a small lobulated projection at the proximal end of the brood pouch. Upon ovulation, the outer follicle is cast off in the ovary and the egg is discharged into the brood pouch. Probably, fertilization takes place in the brood pouch, where the newly ovulated egg is heavily surrounded by sperm. The fertilized egg undergoes cleavage and embryogenesis within the expanded chorion, over which stretched inner follicular cells are attached.

In all species, the brood pouch is a tubular organ lined by the peribranchial epithelium, whose cells are little specialized and are quite similar in appearance to those of other parts. In *P. formosana*, the brood pouch is wholly attached to the mantle. In cross section, the brood pouch appears as a perfect tube, except for a short distance near the distal end where it is broken or discontinuous at a point toward the outer margin (Figs. 2C, D; 3A, B). In *P. japonica*, the brood pouch occupies the immediate posterior end of the peribranchial cavity; thus, a basal part of the branchial wall participates in its formation. Therefore, the brood pouch of this species is situated somewhat posteriorly as compared with that of *P. formosana*. As in *P. formosana*, the brood pouch of *P. japonica* is a perfect tube in its proximal portion; but near the distal end it is discontinuous at a point toward the mantle (Figs. 2E, F; 3C, D). The brood pouch of *P. sagamiensis* initially develops more posteriorly than that of *P. japonica*. It lies below the peribranchial cavity (Figs. 2G, 3E). Around the distal opening of the brood pouch leading to the peribranchial cavity, the epithelial cells are thickened and are able to close the opening (Fig. 3F). These observations, coupled with the external observations described before, clearly show that in *P. formosana* the brood pouch is formed by the growth of a partition wall within the original peribranchial cavity; while in *P. sagamiensis* the brood pouch is formed by an evagination from the floor of the peribranchial cavity into the posterior part of the mantle. The brood pouch of *P. japonica* seems to be formed by a combination of these two ways, with the growth of a partition wall playing the major part.

As described earlier, in *P. sagamiensis* the branchial basket and digestive tract are resorbed soon after the initiation of brooding, and the brood pouch expands, filling up the space formed by their resorption (Fig. 2H). It should be noted that the brain ganglion remains intact throughout the period of brooding.

Ordinary budding or subterminal budding

In every species of *Perophora*, a budding site is located a short distance from the tip of a growing stolon, where a bud arises and develops into a zooid. This ordinary type of budding is here termed subterminal budding. As the stolen elongates, it produces successively new buds. Therefore, so far as the colony grows by subterminal budding, zooids are arranged on the stolon in order of age with the youngest next to the growing tip. Each zooid is situated with the ventral side (*i.e.*, the side of endostyle) directed toward the distal end of the stolon.

The course of blastogenesis in subterminal budding has been well documented by earlier workers (Lefèvre, 1898; Brien and Brien-Gavage, 1927; Deviney, 1934). Consequently, we are not especially concerned with this subject in the present study. Only a few words will suffice. A bud primordium consists of a double vesicle. The outer vesicle is derived from the epidermal wall of the stolen and gives rise to the epidermis of the zooid. The inner vesicle is derived from the septum of the stolon and gives rise to all adult tissues and organs except the epidermis. For the formation

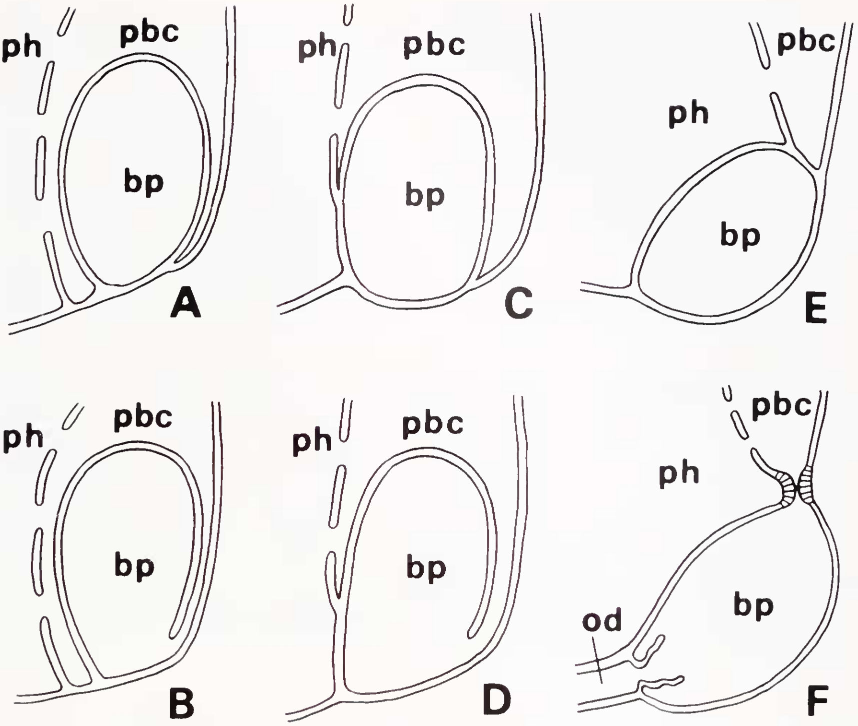

FIGURE 3. Diagrammatic representation of the brood pouch. A: *P. formosana*, cross section through a proximal part of the brood pouch. B: *P. formosana*, cross section through a distal part of the brood pouch. C: *P. japonica*, cross section through a proximal part of the brood pouch. D: *P. japonica*, cross section through a distal part of the brood pouch. E: *P. sagamiensis*, cross section of an early brood pouch. F: *P. sagamiensis*, longitudinal section of an early brood pouch. bp, brood pouch; od, oviduct; pbc, peribranchial cavity; ph, pharynx.

of the inner vesicle and its subsequent organogenesis, lymphocytes in the circulating blood also may be employed (Deviney, 1934; Freeman, 1964). This mode of blastogenesis is generally known as stolonial budding.

The colony is extended by terminal growth and lateral branching. At least in the three species presently examined, the elongation of stolon occurs only near its tip, in the portion distal to the youngest bud. The branching of stolon or the formation of new stolons occurs most vigorously near the distal end of a growing stolon and around the bases of developing buds. In *P. formosana*, when the growing tip of a stolon encounters another stolon of the same colony, the former usually continues to grow crossing over the latter. However, in *P. japonica* and *P. sagamiensis*, stolons of the same colony easily anastomose and fuse together, thus forming a network of stolons.

Dormant stolons

In Shimoda, *P. formosana* undergoes hibernates, as reported briefly by Fukumoto (1971) with *Perophora orientalis*, which is homospecific with *P. formosana* (*cf.* Tokioka, 1953). Every year, all the zooids and buds regressed in late autumn (November), and only stolons survived the winter months (Fig. 4A). The overwin-

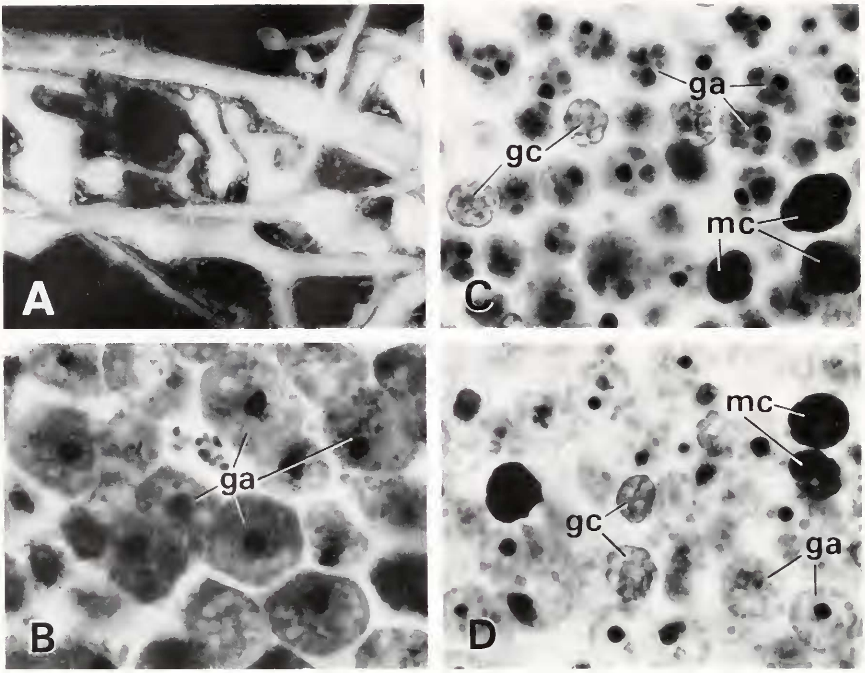

FIGURE 4. Dormant stolons. A: *P. formosana,* external appearance. ×12. B: *P. formosana,* congested blood cells. ×1000. C: *P. japonica,* congested blood cells. ×1000. C: *P. sagamiensis,* congested blood cells. ×1000. ga, granular amoebocytes; gc, green cells; mc, morula cells.

tering stolons were opaque and yellowish, owing to the accumulation of blood cells. Such packed opaque stolons, in which development is inhibited until the environment becomes more favorable, are generally termed dormant stolons in this paper. The dormant stolons were often constricted into bulges, where the tunic grew thicker and harder than usual. The bulges might be isolated, singly or in connection of several bulges, from other stolons by disintegration of the latter, though this was not an inevitable process.

The overwintered dormant stolons of *P. formosana* produced new blastozooids from late April to early May. The new blastozooids were often budded directly from old stolons; but the bulged portions of stolons first elongated new stolonic outgrowths, which then produced subterminal buds.

The colonies of *P. sagamiensis* showed a tendency to hibernate, though their behavior during winter differed from year to year. In some years, almost all zooids regressed in winter, and stolons survived the winter and regenerated zooids in late spring as in *P. formosana.* However, in some other years many zooids escaped regression and survived the winter.

Generally, *P. japonica* colonies continued to grow with functional zooids throughout the year. However, in midsummer (August) their zooids occasionally regressed to disappear from limited areas, leaving dormant stolons. Such dormant stolons sent out slender new stolons within a few weeks or so. Thus, this species shows a slight tendency of estivation instead of hibernation, at least in Shimoda.

Blood cells of *Perophora viridis* have been divided into several morphologically distinct classes (George, 1926; Freeman, 1964; Overton, 1966). In histological preparations, the dormant stolons of all species studied are filled with blood cells. The most abundant cells are spherical and contain coarse eosinophilic granules in the cytoplasm. These cells function as trophocytes and can be identified as granular amoebocytes in the terminology of the previous authors, though their size and appearance are somewhat variable with the species (Fig. 4B–D). In addition to the granular amoebocytes, berry-like cells consisting of bright yellowish-green bodies, the green cells, are relatively common in the dormant stolons of all species. Morula cells, which are intensely stained with hematoxylin and eosin, are found in the dormant stolons of *P. japonica* and *P. sagamiensis;* but this cell class is absent from the blood of *P. formosana.*

Terminal budding in Perophora japonica

It was found that in *P. japonica* the distal tip of a growing stolon turns into a kind of asexual reproductive body, which is eventually severed from the stolon and drifts away to found a new colony. This type of asexual reproduction has never been reported before, and is termed "terminal budding" in this paper.

Terminal buds at various stages of formation are shown in Figure 5A. In a "normal" stolon, the growing tip is fully attached to the substratum by the lower side, and the septum usually terminates in a flattened vesicle (Fig. 6A). At the start of terminal budding, a distal portion of the stolon rises up off the glass plate, losing contact with the substratum, and grows into a hyperplastic bulge. Then, the terminal

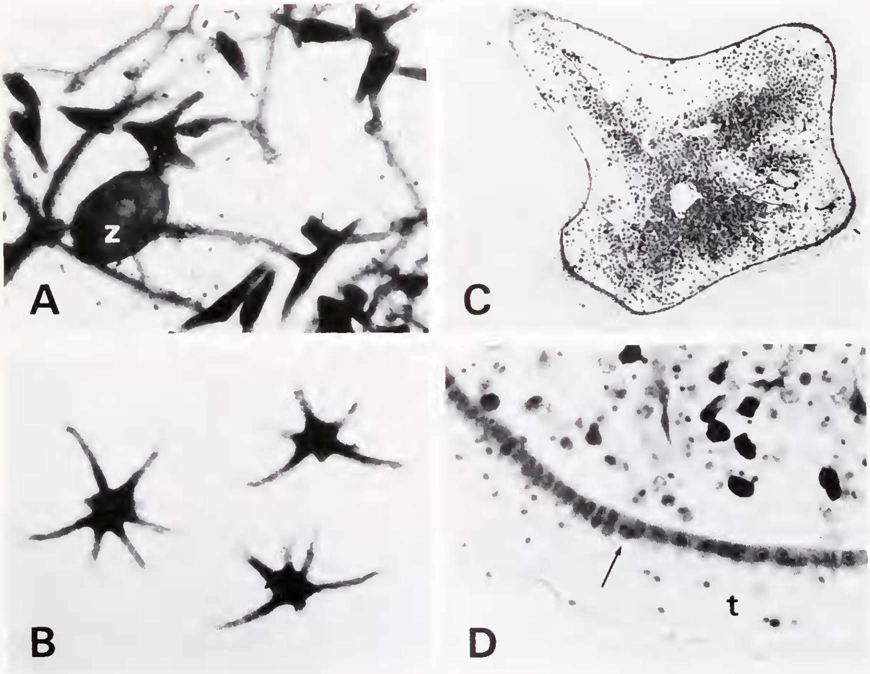

FIGURE 5. Terminal buds of *P. japonica.* A: Terminal buds at various stages of their formation. Extremely thin stolons are of hydrozoan colonies. z, zooid. ×8. B: Isolated terminal buds. ×8. C: Section of an early terminal bud. Blood cells, mostly granular amoebocytes, are accumulated in the lumen; septal walls are branched. ×70. D: Part of an early terminal bud, showing thickened epidermal cells and a mitotic figure (arrow). t, tunic. ×400.

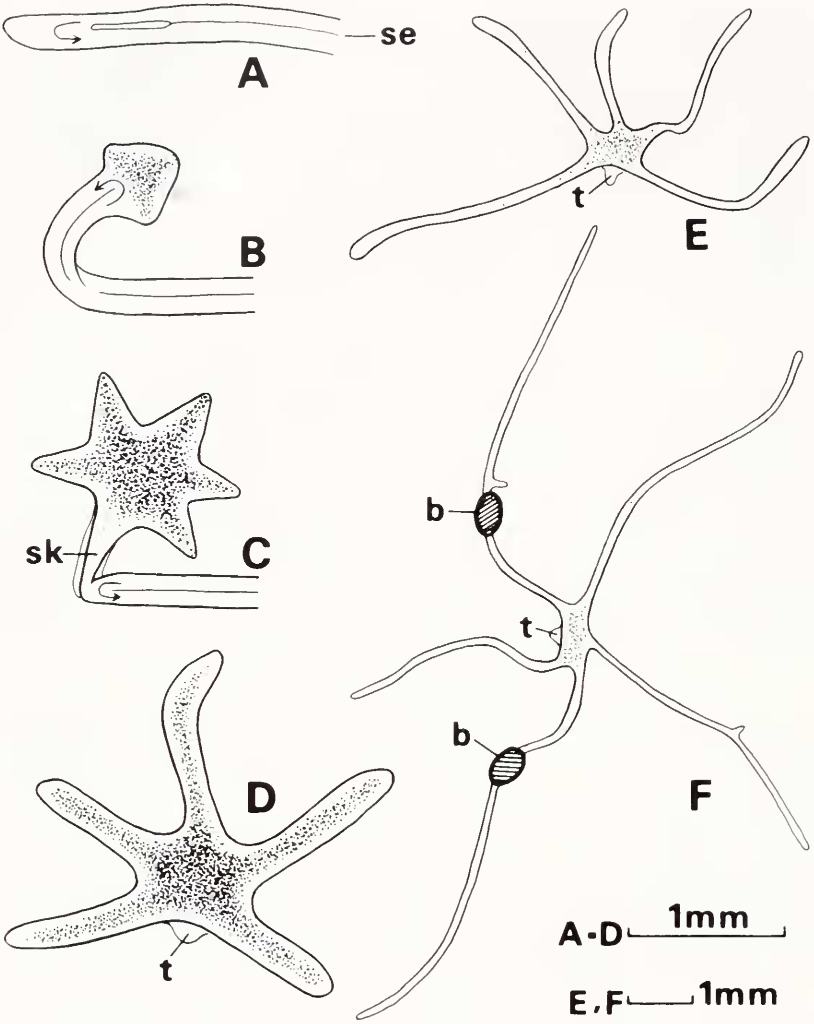

FIGURE 6. Diagrammatic representation of the formation and development of the terminal bud in *P. japonica.* A: Normal growing tip of a stolon. B: Early stage of terminal budding. C: Terminal bud before isolation. D: Terminal bud soon after isolation. E: One day after isolation. F: Two days after isolation. b, subterminal bud; se, septum; sk, stalk; t, tunic as a vestige of the stalk.

bulge, containing the terminal vesicle of the septum, expands fanwise (Fig. 6B). Subsequently, it develops epidermal projections, 3 to 6 in number, along its margin (Fig. 6C). These projections are stolon primordia. During these processes, the terminal bud becomes opaque by the accumulation of blood cells; and, the peduncular portion or stalk that connects the bud proper and the basal creeping stolon curves more and more upward until the bud stands straight or even until it turns upside down. The blood flow in the stalk ceases, and at last the bud becomes fully severed from the stolon. The isolated terminal bud is discoidal and stellate in outline (Figs. 5B, 6D); its stolons are now elongating. In the summer season (at about 25°C), it takes about 2 days from the initiation of swelling of the stolon tip to the isolation of it as a mature terminal bud. A stolon forms only one terminal bud at a time, yet it can produce many consecutively. Both terminal and subterminal budding can occur simultaneously in the same stolon.

In sections, the terminal bud consists of tunic, epidermis, septal walls, and blood; no sign of blastogenesis is found in it. The majority of the blood cells are granular amoebocytes, and some others are green cells and morula cells. The mesenchymatous septum is highly branched (Fig. 5C). The epidermis is thickened in the stolon primordia, where mitotic figures are common during their formation (Fig. 5D).

A free terminal bud may be carried away by water currents to some distance. Its surfaces are very sticky; therefore, it adheres to almost any solid substance that it may chance to encounter. After attaching to a substratum, the bud elongates its stolons rapidly, forming a new colony without zooids (Fig. 6E). Then, after the stolons have grown to considerable lengths, they develop buds which grow into zooids. The number of the initial buds is usually less than three in a colony; a stolon can produce only a single bud at a short distance from the tip (Fig. 6F). That is, the appearance of blastozooids in this colony is effected by ordinary subterminal budding. In running sea water at about 25°C, the initial blastozooids develop into vesicular buds in about 2 days after isolation of the terminal bud; and about 2 more days pass before they differentiate into functional zooids. In standing sea water, these processes proceed more slowly.

A slow irregular circulation of blood is seen in the stolons growing from the terminal bud, even before the appearance of the initial buds or blastozooids. This blood flow is clearly caused by the contraction of stolonic epidermis. At first, each stolon has only one channel. Later, as nutritive blood cells are consumed, the stolon acquires two channels, developing a septum in the lumen. In such a stolon, blood only flows still back and forth, with a 2–3 min cycle. Blood flow reversals are usually not synchronized in the stolons of the same colony. Even when the hearts of the initial zooids start beating, blood flow in opposite directions between two channels as in normal stolons is limited to short ranges near the zooids. Somewhat similar circulation of blood which is effected by the contraction of epidermal cells has been observed by Huxley (1921) with isolated zooid-stolon preparations of *Perophora viridis*, especially during the later stages of zooid resorption.

In large colonies grown on glass plates, terminal budding took place vigorously throughout the non-breeding period of year. During the breeding season, gonads first appeared in some zooids located in the older parts of a colony. In such a colony, terminal budding continued only in those stolons which were distant from the zooids with mature gonads. As more and more zooids of the colony came to engage in sexual reproduction, terminal budding ceased in the colony. Even during the peak period of sexual reproduction of this species, under our culture conditions, some colonies remained showing little sexual activity. Such colonies continued forming terminal buds.

More information concerning the relation between terminal budding and sexual reproduction and that between terminal budding and colony age was obtained by the following observations. During August and September 1981, we observed two colonies founded by terminal buds. Thirty-five days after the settlement of the terminal buds, one colony had 27 functional zooids and 9 developing terminal buds, and the other colony had 10 functional zooids and 4 early terminal buds. In both colonies, no previous formation of terminal buds was noted. Similar observations were performed also during the early part of the breeding season, from October to November 1980, with seven colonies derived from terminal buds. Twenty-nine days after the settlement of the terminal buds, they were found bearing 57–90 functional zooids. Immature gonads were found in small numbers of zooids of only two colonies, but no terminal bud was found in any colony. After 43 days from the settlement, every colony had 240–420 functional zooids, a considerable number of which bore gonads. In four colonies, only 4–6 terminal buds were counted; and in three colonies no terminal bud was found. A week later, in all colonies many zooids had gonads and some of them carried even growing embryos; while none of the colonies bore a terminal bud. Similar results were obtained with colonies founded by metamorphosis of larvae. From October 1979 to January 1980, correspondingly to the peak period of sexual reproduction, we observed several such colonies. In this series, none of the colonies produced a terminal bud during the observation period of about 40 days. From these results, it is concluded that terminal budding is suppressed by sexual reproduction. Furthermore, these results suggest that juvenile colonies lack the capacity of terminal budding.

No evidence of terminal budding was found in *P. formosana* and *P. sagamiensis.*

Replacement budding in Perophora sagamiensis

As already described, the zooids of this species invariably degenerate after sexual reproduction. Often, but not always, such degenerated zooids are replaced by new blastozooids.

When a degenerating zooid appears as a minute knob, or sometimes after it has completely disappeared, a new bud appears on the stolon at the place where the original zooid has previously been located (Fig. 7). Generally, some accumulation of blood cells, largely granular amoebocytes, is found in the stolon near the bud. This bud develops into a functional zooid, with the same polarity as that of the original zooid. This type of budding is here termed "replacement budding" as distinct from subterminal budding; though, as judged by external observation, blastogenesis is alike in both types.

Replacement buds are very common during the sexual breeding season. They are also found during the remainder of the year, but are few in number probably

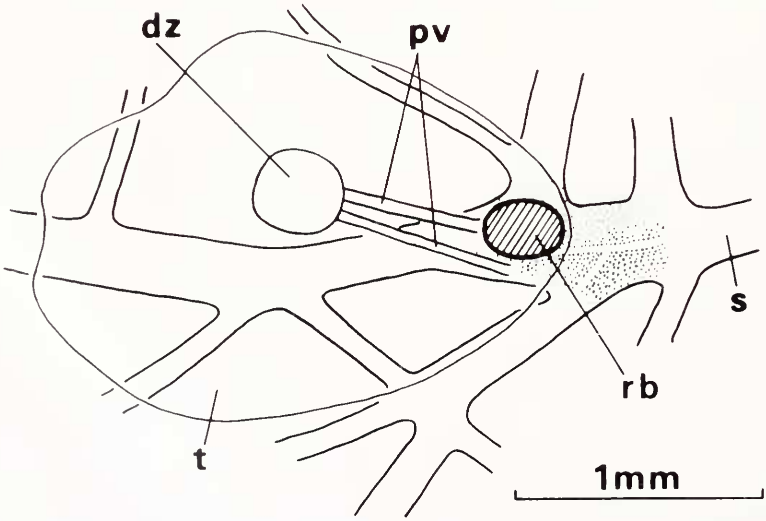

FIGURE 7. Replacement budding in *P. sagamiensis.* dz, degenerating zooid; pv, peduncular vessels of the degenerating zooid; rb, replacement bud; s, stolon; t, tunic ghost of the degenerating zooid.

because a relatively small number of zooids degenerate by physiological senescence in this period.

No sign of replacement budding is seen in *P. formosana* and *P. japonica.*

Discussion

Perophora is an enterogonid ovoviviparous ascidian. The gonads are located on the left side, within the first intestinal loop. The sperm duct opens into the cloacal cavity; while the oviduct opens at the postero-dorsal corner of the right peribranchial cavity, discharging the eggs into the brood pouch consisting of the peribranchial epithelium. These features are also shared with *Ectanascidia* (see Simkins, 1924; Berrill, 1932).

The position of the brood pouch and the behavior of the mother zooid during brooding are different in different species. In *P. formosana* the entire brood pouch is attached to the mantle, but in *P. japonica* both the mantle and the branchial wall participate in the formation of the brood pouch. In these species, the zooid generally remains functional during and after sexual reproduction. On the other hand, in *P. sagamiensis* the brood pouch initially develops at a level posterior to the original peribranchial cavity. The branchial basket and digestive tract disintegrate soon after the onset of brooding, then the brood pouch expands into the space formerly occupied by the branchial basket. During the brooding period, the zooid remains as a sac with the heart and ganglion intact. After the completion of brooding, the zooid is wholly resorbed. A similar manner of brooding occurs in a pleurogonid colonial ascidian, *Botryllus primigenus* (Mukai, 1974, 1977).

The term "bud" or "budding" has been used in various senses in the literature of ascidian reproduction (*cf.* Berrill, 1935). A bud may be broadly defined as an asexually produced reproductive body, without reference to the presence or absence of the primordium of a blastozooid. Functionally, the buds defined above may be divided into three types: 1) buds which are concerned with the growth of the colony, 2) buds which are destined to found new colonies, and 3) buds whose function is primarily to survive adverse conditions. In the genus *Perophora,* these three types of buds are all found. The first type is represented by the subterminal bud, and the replacement bud may be considered a special form of this type. The terminal bud belongs to the second type, while the dormant stolon belongs to the third type.

In a more restricted sense, a bud may be defined as a rudimentary stage of a blastozooid. In this sense, the term budding is nearly equivalent to blastogenesis. In *Perophora,* only a single type of blastogenesis is known. That is, a blastozooid is always formed by the so-called stolonial budding. It is generally admitted that most colonial ascidians are given a single mode of blastogenesis. Only *Botryllus* and *Botrylloides* are known to be able to propagate by two different kinds of blastogenesis, *i.e.,* by palleal and vascular budding (Oka and Watanabe, 1957, 1959).

So far, only subterminal budding has been described in the natural growth of the colony in all species of *Perophora.* However, in *P. sagamiensis* new zooids are also formed by replacement budding, in addition to the ordinary subterminal budding. Consequently, in this species, the age-graded arrangement of zooids along the stolon which was once established by subterminal budding is put in disorder. Probably, the capacity of replacement budding may have developed in accordance with the habit of zooid degeneration following sexual reproduction.

In Shimoda, *P. formosana* undergoes typical hibernation. *P. sagamiensis* also displays hibernation to a lesser degree, while *P. japonica* shows a slight tendency of estivation. During hibernation or estivation, the colony survives in the form of dormant stolons filled with granular amoebocytes. Such stolons, of course, contain the mesenchymatous septum as a formative tissue, but blastogenesis is arrested until the environment becomes more favorable. The regression of zooids and subsequent formation of dormant stolons can also be induced experimentally under various adverse conditions in *Perophora listeri* (Brien and Brien-Gavage, 1927) and *P. viridis* (Huxley, 1921; Barth and Barth, 1966). Thus, the ability to produce dormant stolons appears to be potentially present in every species of *Perophora.* Somewhat similar dormant buds have long been known in the Clavelininae, in which new blastozooids develop from terminal ampullae of the so-called stolons or posterior extensions of the abdomens of individual zooids, after their isolation following the resorption of the zooids at the end of the sexual breeding season (*cf.* Berrill, 1950).

Our terminal bud was first described and illustrated as "a stellate structure" in the original description of *P. japonica* by Tokioka (1953). He considered its formation a diagnostic character of this species, but did not consider its function.

The terminal bud is similar to the dormant stolon. It is derived from a stolon following the accumulation of granular amoebocytes and in the absence of a zooid primordium. However, the dormant stolon is formed under unfavorable environmental conditions, and has a remarkable resting ability. By contrast, the terminal bud is formed only in an actively growing stolon. No evidence of arrested development of the terminal bud has been obtained. Terminal budding occurs concomitantly with subterminal budding, but is clearly suppressed by sexual reproduction. Further study is needed to know the regulatory mechanisms among these different modes of reproduction.

In the asexual reproduction of ascidians, only a few examples of dispersal mechanisms are known. Probably, the terminal bud of *Perophora japonica* is best compared with the planktonic-type bud of *Polyzoa vesiculiphora,* a polystyelid ascidian. In this species, two main types of palleal buds, stolonic and planktonic, are formed. The planktonic-type bud is congested with granular amoebocytes, isolated from the mother zooid, and may be carried away by water currents to found a new colony elsewhere (Watanabe and Tokioka, 1972; Fujimoto and Watanabe, 1976a, b). However, the planktonic-type bud of *Polyzoa* and the terminal bud of *Perophora* differ significantly. The former is essentially a zooid primordium that grows directly into a functional zooid, developing new test vessels from its ventral epidermis. By contrast, the terminal bud first grows stolons, which then produce zooids.

Terminal budding is apparently peculiar to *P. japonica.* This species is said to be very closely allied to *P. viridis, P. listeri,* and *P. banyulensis* (Tokioka, 1953). For similarities of the latter three species, see also Van Name (1945) and Berrill (1950). It is uncertain whether terminal budding also occurs in these species.

P. formosana differs from *P. japonica* and *P. sagamiensis* in many points. In *P. formosana,* a young zooid may have four rows of stigmata, but a full-grown zooid has five rows of stigmata with the first and second rows imperfectly separated; while the zooids of *P. japonica* and *P. sagamiensis* have strictly four rows of stigmata. *P. formosana* has a non-lobulated massive testis and an ovary situated at a distance from the testis; its blood lacks morula cells. By contrast, both *P. japonica* and *P. sagamiensis* commonly have a lobulated testis and an ovary situated at the proximal end of the sperm duct; their blood contains morula cells. Moreover, in *P. formosana* (= *P. orientalis*), fusion of stolons even of the same colony does not occur in natural growth; but experimental fusion of stolon-pieces is easily attained without reference to their origin, indicating the absence of colony specificity in tissue compatibility (Mukai and Watanabe, 1974). On the other hand, in *P. japonica* and *P. sagamiensis,* stolons of the same colony always fuse in natural growth; but those of different colonies sometimes fuse and sometimes do not, thus indicating the presence of colony specificity (Koyama and Watanabe, 1981, 1982). These differences probably reflect phylogenetic relationships. *P. formosana* seems to be more primitive than the other two species.

In *P. sagamiensis*, the testis is lobulated but forms as an irregular mass. This fact suggests that this species is intermediate between *P. formosana* and *P. japonica* (*cf.* Tokioka, 1953). However, the formation of the brood pouch and the mode of brooding clearly show that *P. sagamiensis* is more advanced than *P. japonica*. It may be concluded that the faculty of terminal budding in *P. japonica* and that of replacement budding in *P. sagamiensis* have been acquired independently by respective species after their separation.

We believe that similar studies of many other species of the Perophoridae will be very helpful in elucidating the phylogenetic relationships within the Perophoridae and those between this and related families.

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